

THIOREDOXIN *h* CONSTRUCTS FOR TRANSFORMATION

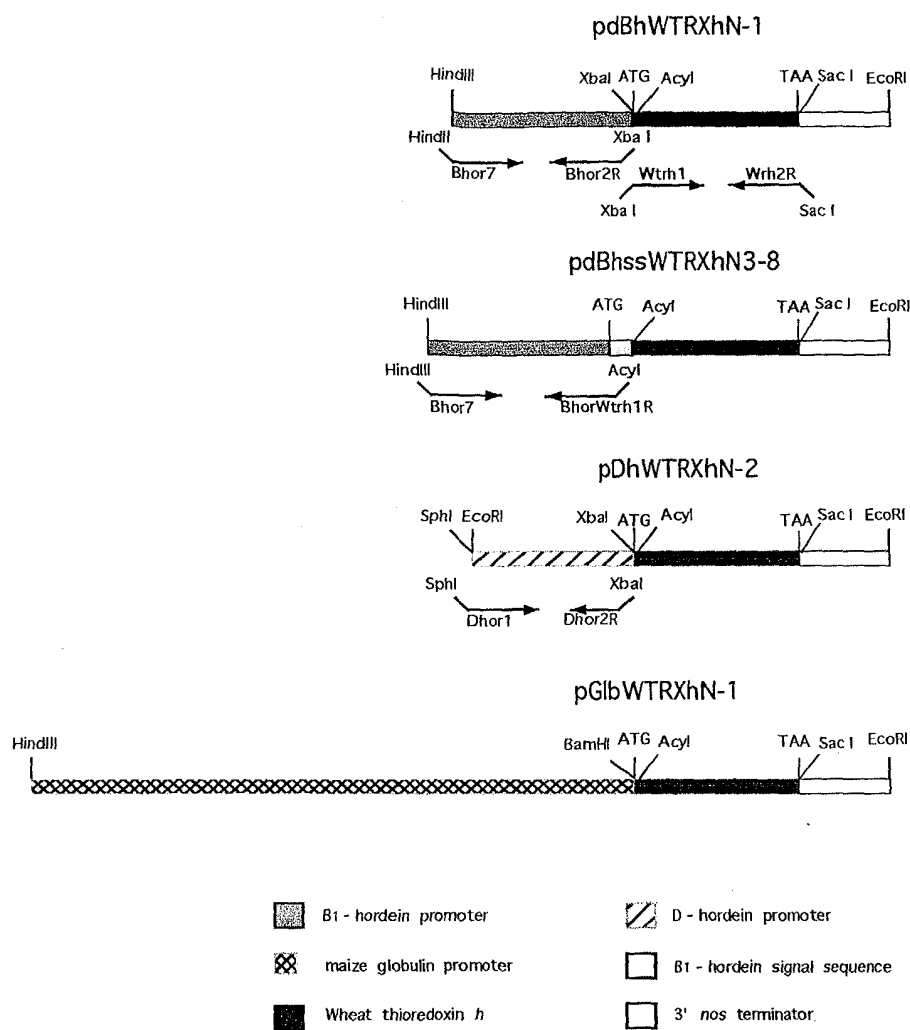
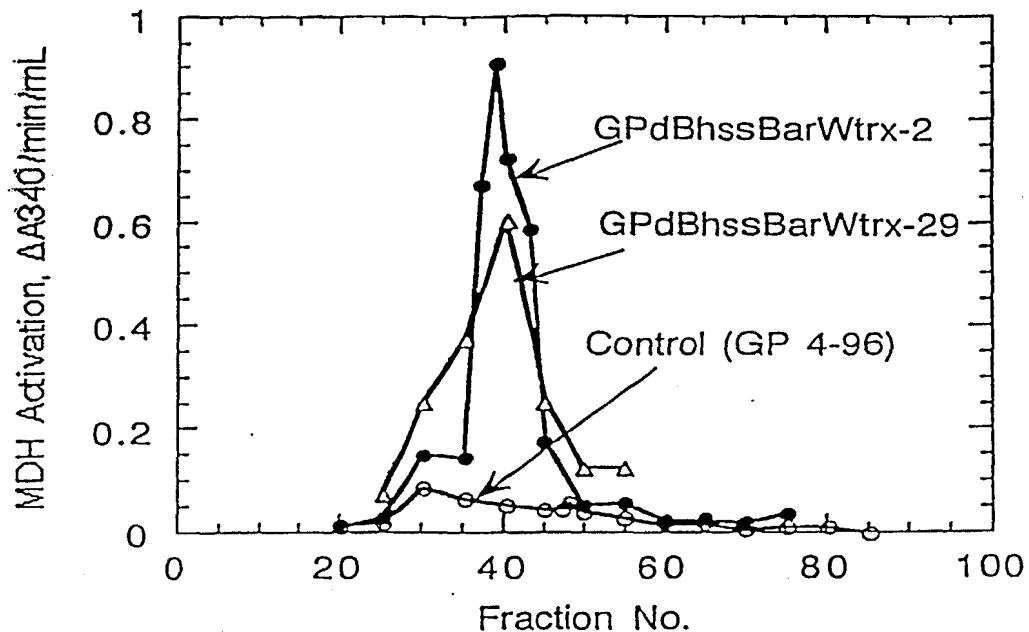


Figure 1

00020-11906560

Sephadex G-50 Activity Profile of Thioredoxin from Barley Grains (+MDH)



Sephadex G-50 Elution Profile of Proteins from Three Barley Grains

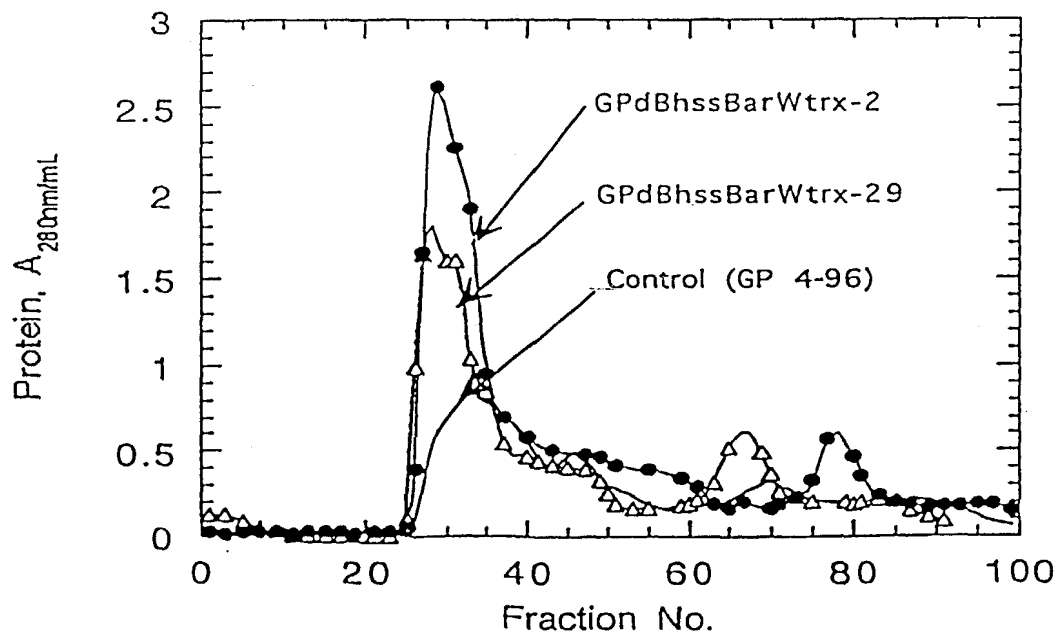


Figure 2

Effect of Heat Treatment on Thioredoxin Activity in Crude Extracts from Grain of Control (C) and Transformed (T) Barley

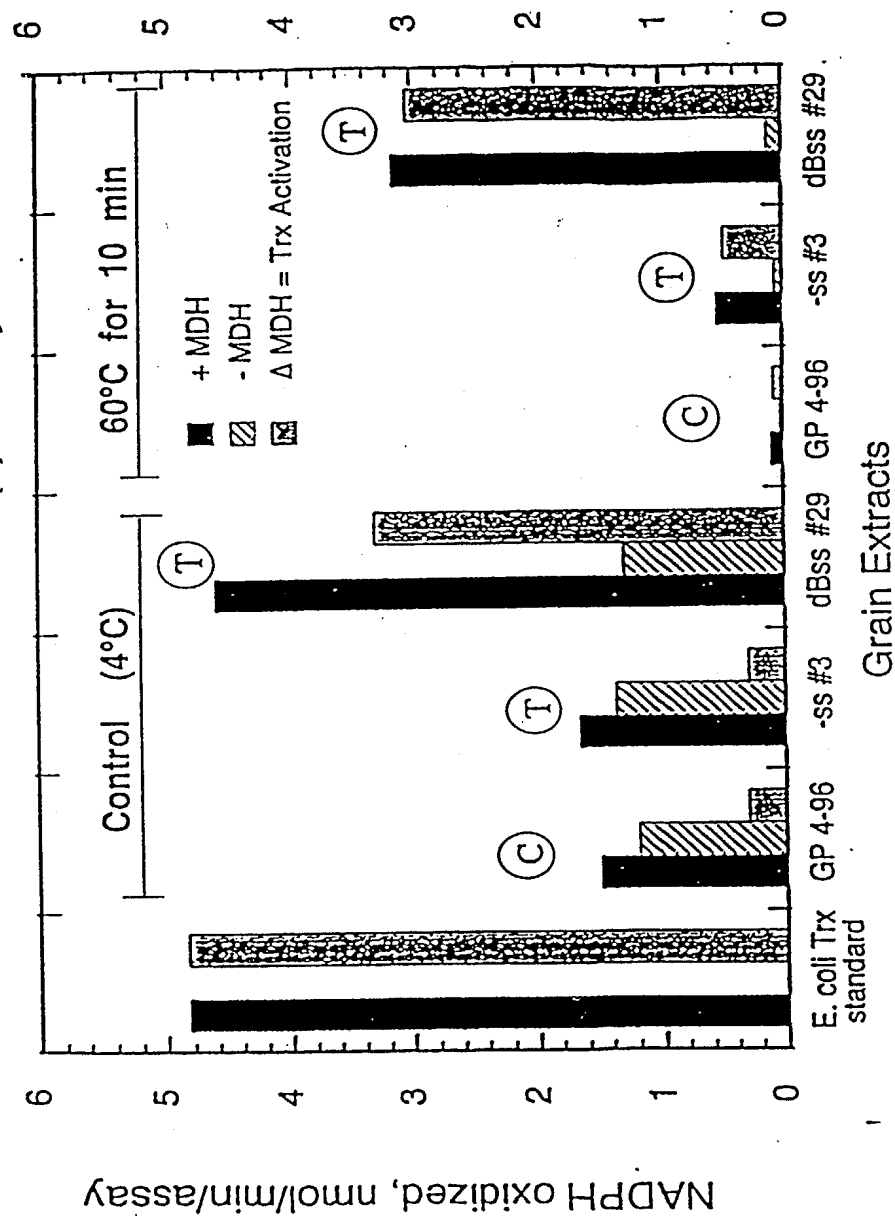


Figure 3

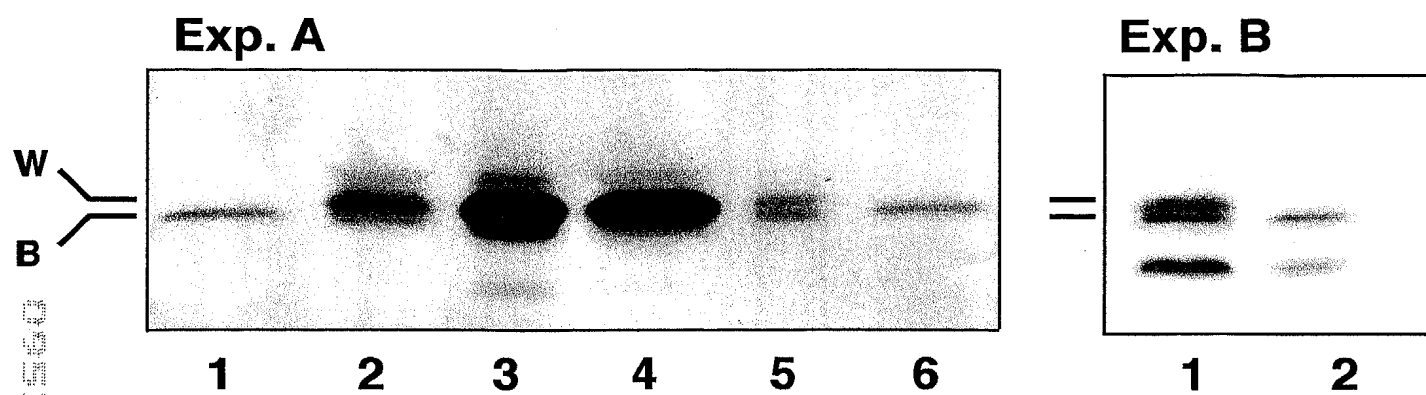
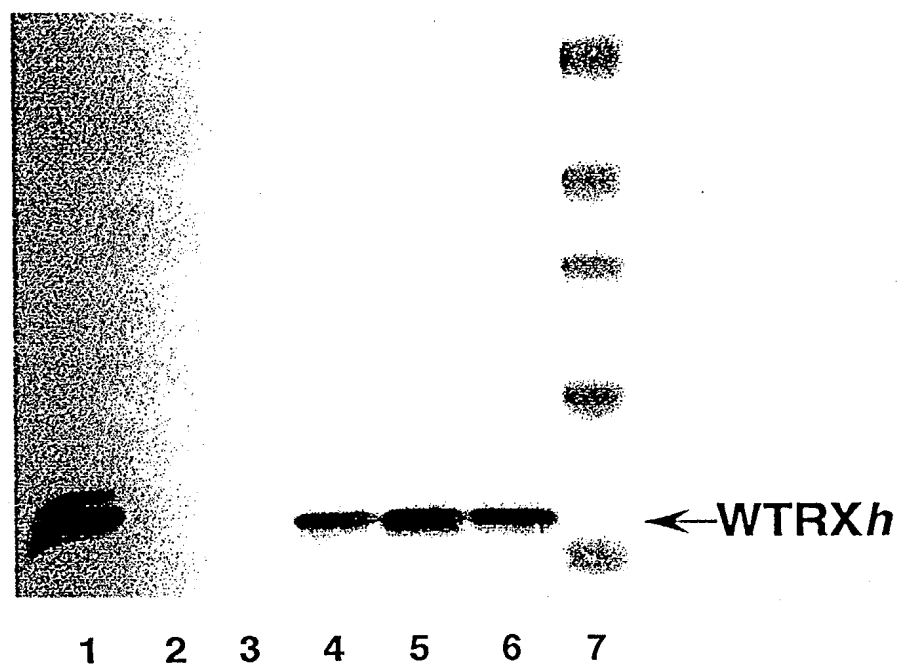


FIGURE 4

Western Blot Analysis of Barley Grain Transformed with Wheat Thioredoxin

SDS-PAGE: cv. Golden Promise



1. Wheat germ thioredoxin
2. Control (GP 4-96), nontransformed
3. Control, null segregant (GPdBssBarWtrx-29-11-10)
4. Transformed, heterozygous line (GPdBssBarWtrx-29)
5. Transformed, homozygous line 1 (GPdBssBarWtrx-29-3)
6. Transformed, homozygous line 2 (GPdBssBarWtrx-29-3-2)
7. Prestained standards

FIGURE 5

AAGCTTTAACAACCCACACATTGATTGCAACTTAGTCCTACACAAGTTTTCCATT
 CTTGTTTCAGGCTAACAACCTATACAAGGTTCCAAAATCATGCAAAAGTGATGC
 TAGGTTGATAATGTGTGACATGTAAAGTGAATAAGGTGAGTCATGCATACCAAA
 CCTCGGGATTICTATACITTTGTGTATGATCATATGCACA'ACTAAAAGGCAACTTT
 GATTATCAATTGAAAAGTACCGCTTGTAGCTTGTGCAACCTAACACAATGTCCA
 AAAATCCATTTGCAAAAGCATCCAAACACAATTGTTAAAGCTGTTCAAACAAAC
 AAAGAAGAGATGAAGCCTGGCTACTATAAATAGGCAGGTAGTATAGAGATCTA
 CACAAGCACAAGCATCAAAACCAAGAAACACTAGTTAACACCAATCCACTATGA
AGACCTTCCTCATCTTTGCACTCCTCGCCATTGCGGCAACAAGTACGATTGCA

FIGURE 6

CTTTCGAGTGCCCGCCGATTTGCCAGCAATGGCTAACAGACACATATTCTGCC
 AAAACCCCAGAACATAATCACTTCTCGTAGATGAAGAGAACAGACCAAGAT
 ACAAACGTCCACGCTTCAGCAAACAGTACCCCGAAGTACTAGGATTAAGCCGAT
 TACGCGGCTTTAGCAGACCGTCCAAAAAACTGTTTTGCAAAGCTCCAATTCC
 TCCTTGCTTATCCAATTTCTTTTGTGTTGGCAAAGTCACTTGTCCAACCGATT
 TTGTTCTTCCCGTGTTTCTTCTTAGGCTAACTAACACAGCCGTGCACATAGCC
 ATGGTCCGGAATCTTCACCTCGTCCCTATAAAAGCCCAGCCAATCTCCACAAT
 CTCATCATCACCGAGAACACCGGAGAACCAAAAAGTACTAGAGATCAATTCATTG
 ACAGTCCACCGAGATGGCTAAGCGGCTGGTCCTCTTTGTGGCGGTAAATCGTC
GCCCTCGTGGCTCTCACCACCGCT

FIGURE 7

FIGURE 8

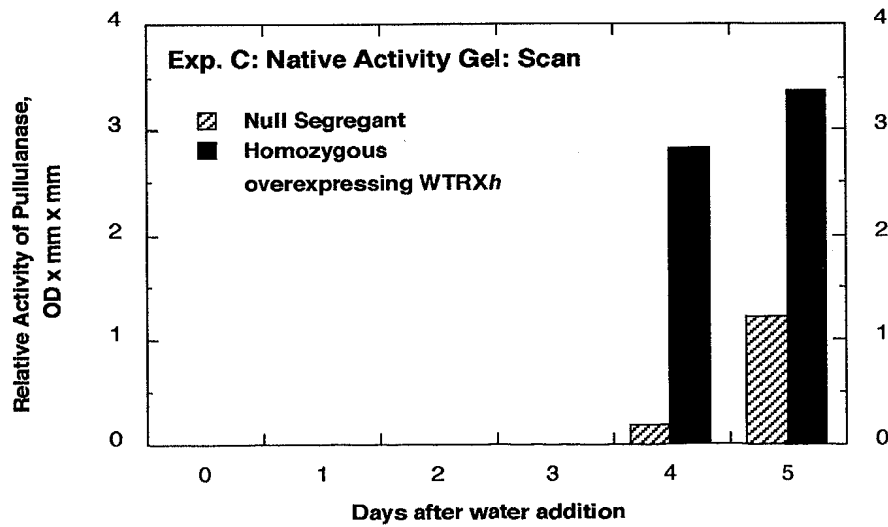
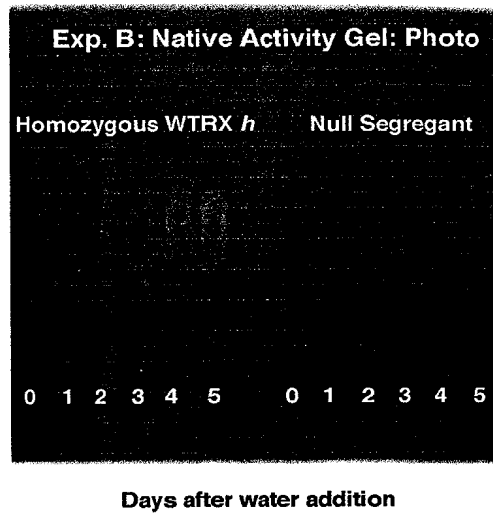
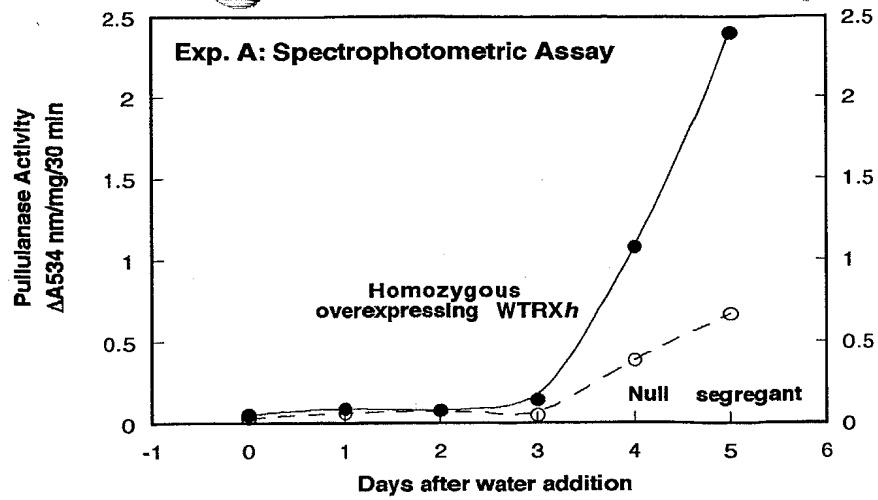


FIGURE 9

Abundance of
 α -amylases

0 1 2 3 4 5 6 7

A

Total Amylase
Activity

Null Segregant

B

0 1 2 3 4 5 6 7

C

Trx-overexpressed

D

006260-19886560

Figure 10

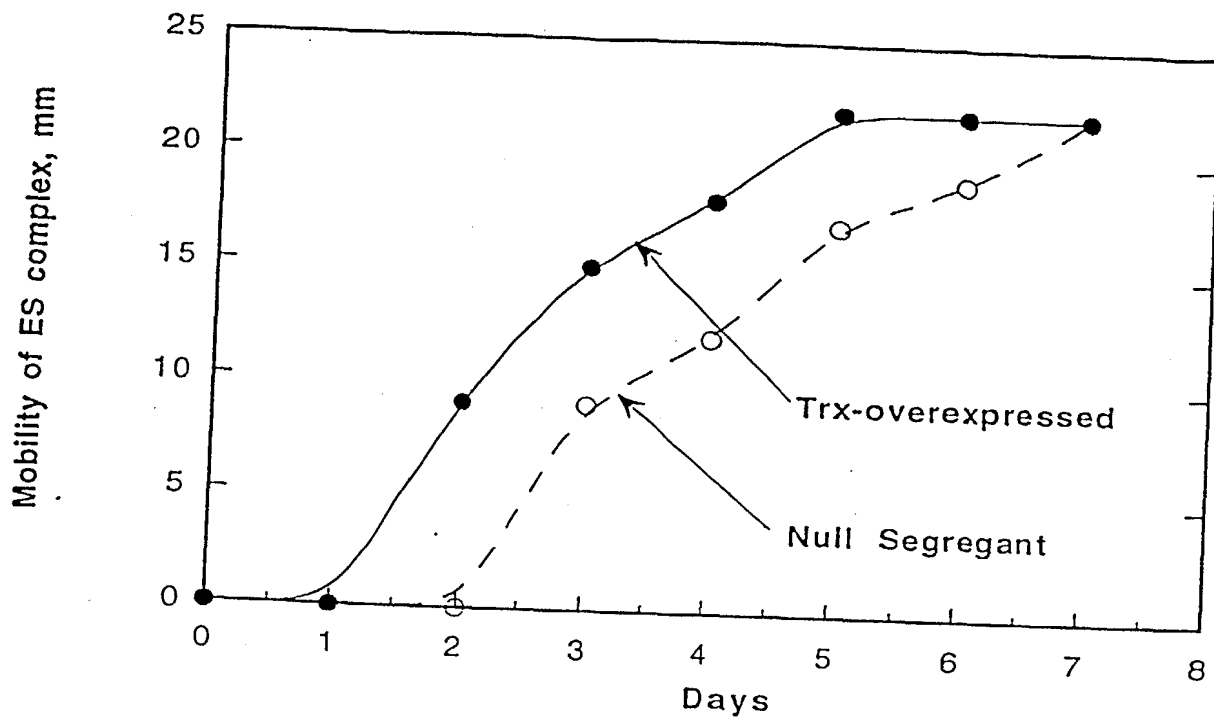


FIGURE 11A

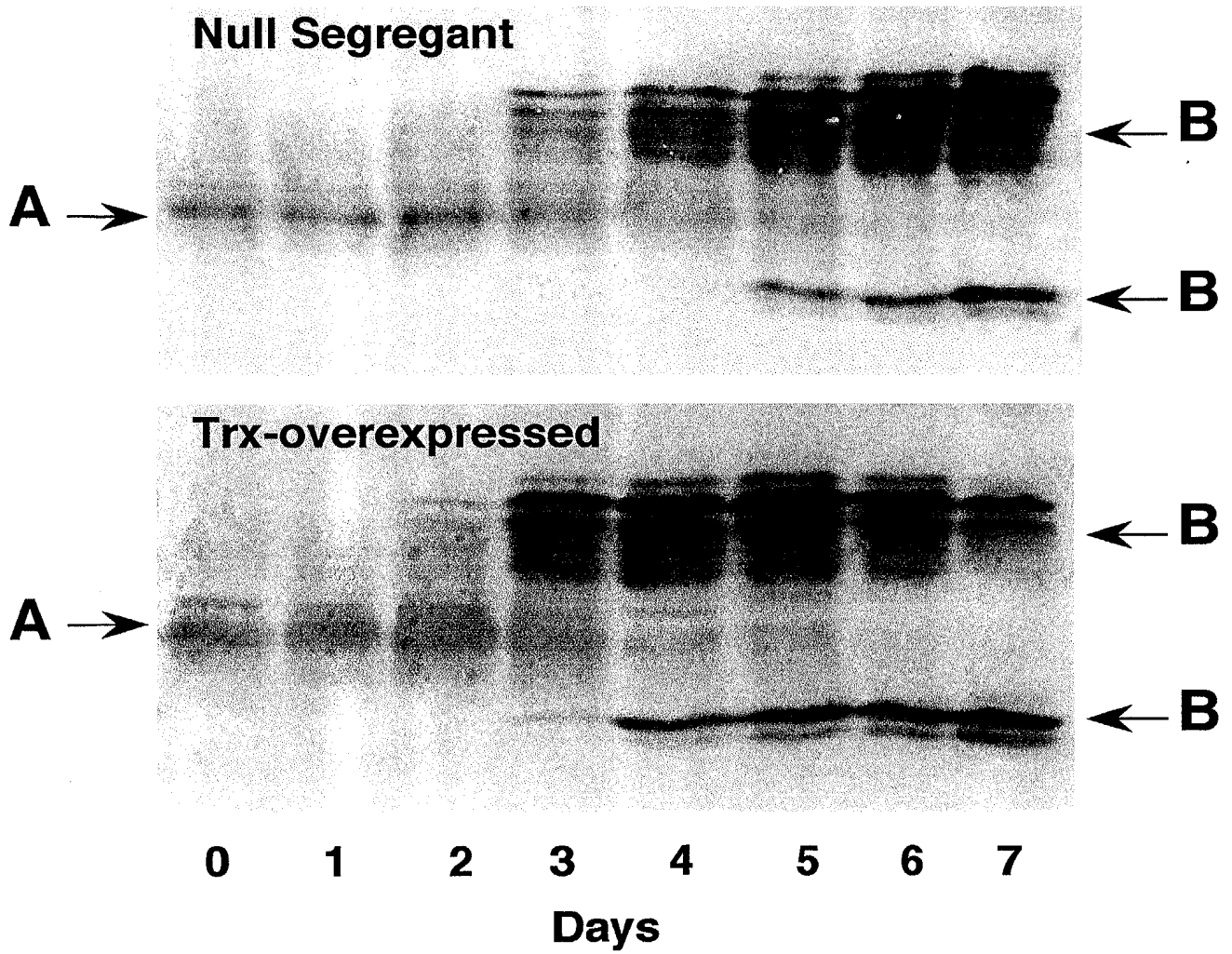


FIGURE 11B

DNA CONSTRUCTS FOR TRANSFORMATION

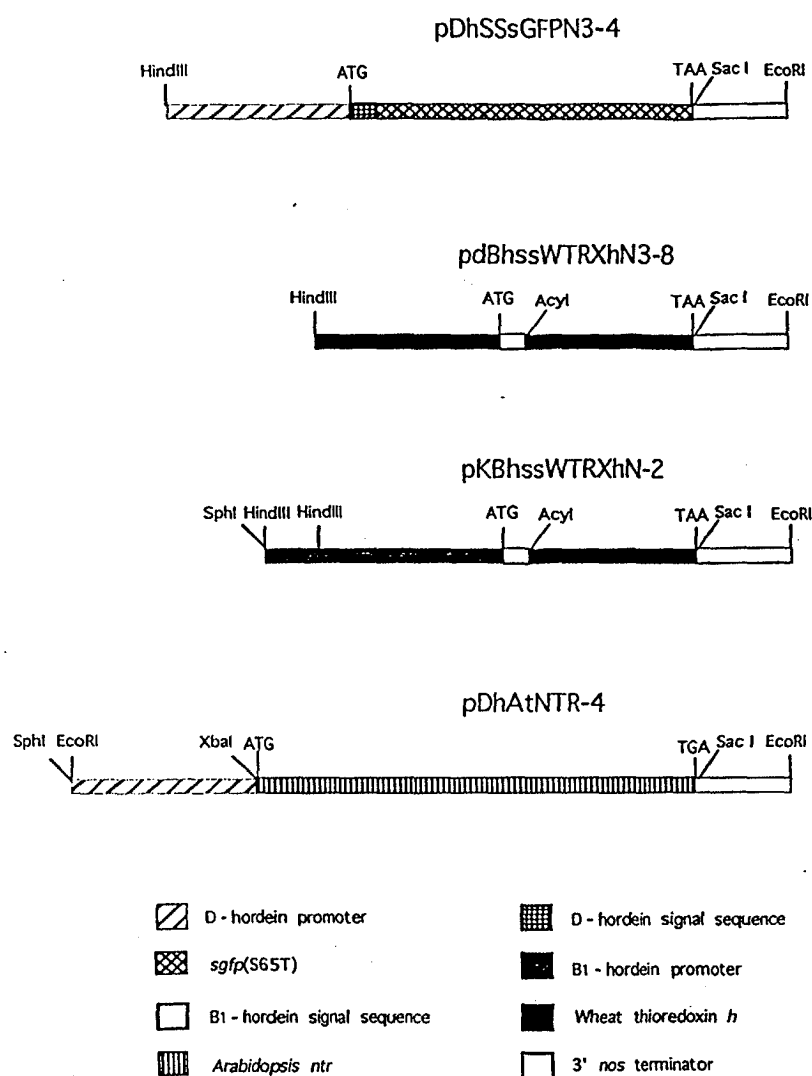


Figure 12

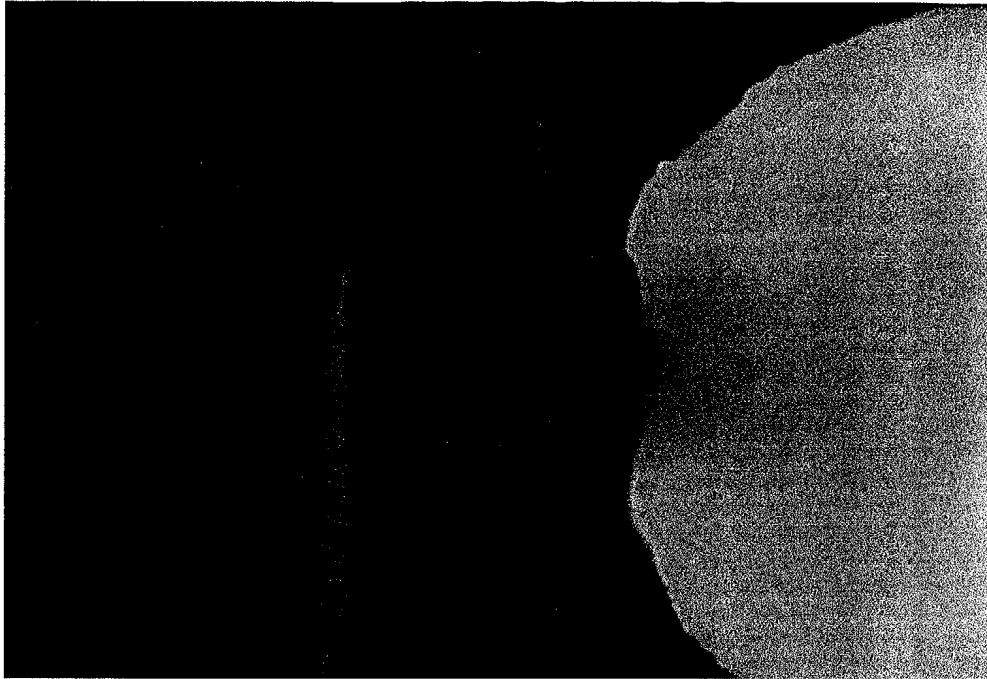


Figure 13

100bp ladder

AZHptWTR-1-25-11

AZHptWTR-1-16-1

AZHptWTR-1-16-2

AZHptWTR-1-16-3

AZHptWTR-1-16-4

AZHptWTR-1-16-5

AZHptWTR-1-24-1

AZHptWTR-1-24-2

AZHptWTR-1-24-3

AZHptWTR-1-24-4

AZHptWTR-1-24-5

(+) Control

0.14-kb
wtrxh

Figure 14

A

Lane Label	Protein Variant
BWHptWtx-1	BWHptWtx-1
BW (nontransgenic)	BW (nontransgenic)
AZHptWtx-1	AZHptWtx-1
AZHptWtx-11	AZHptWtx-11
AZHptWtx-21	AZHptWtx-21
AZ (nontransgenic)	AZ (nontransgenic)
YRHptWtx-1	YRHptWtx-1
YR (nontransgenic)	YR (nontransgenic)
Wheat Germ	Wheat Germ

B

Lane Label	Genotype
Wheat Germ	Wheat Germ
YRHptWtx-1-2-1	null
YRHptWtx-1-2-2	null
YRHptWtx-1-2-3	null
YRHptWtx-1-1-1	homozygous
YRHptWtx-1-1-2	homozygous
YRHptWtx-1-1-3	homozygous
YRHptWtx-1-1-4	homozygous
YRHptWtx-1-1-5	homozygous
YRHptWtx-1-1-6	homozygous
YRHptWtx-1-1-7	homozygous
YRHptWtx-1-1-8	homozygous
YRHptWtx-1-1-9	homozygous

Figure 15

Figure 16

Effect of Thioredoxin Reduction on Digestion of
Wheat Glutenins by Trypsin

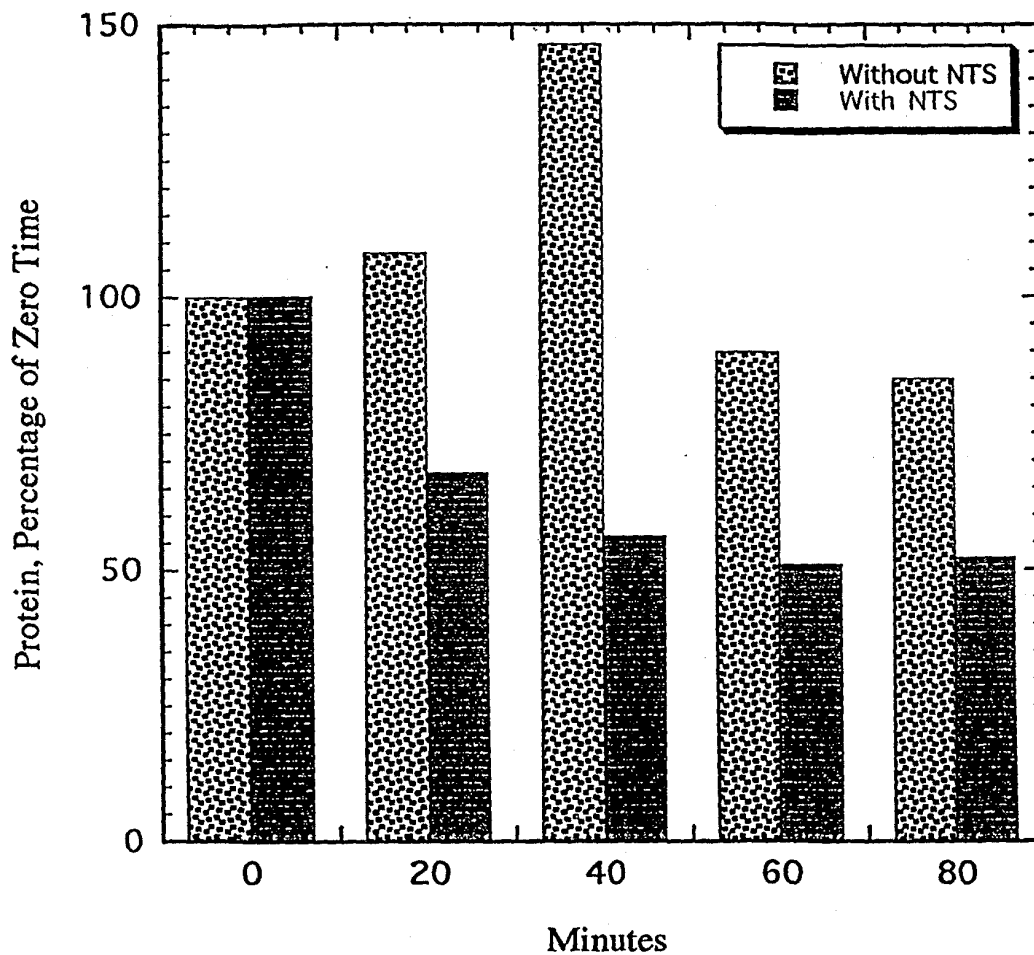
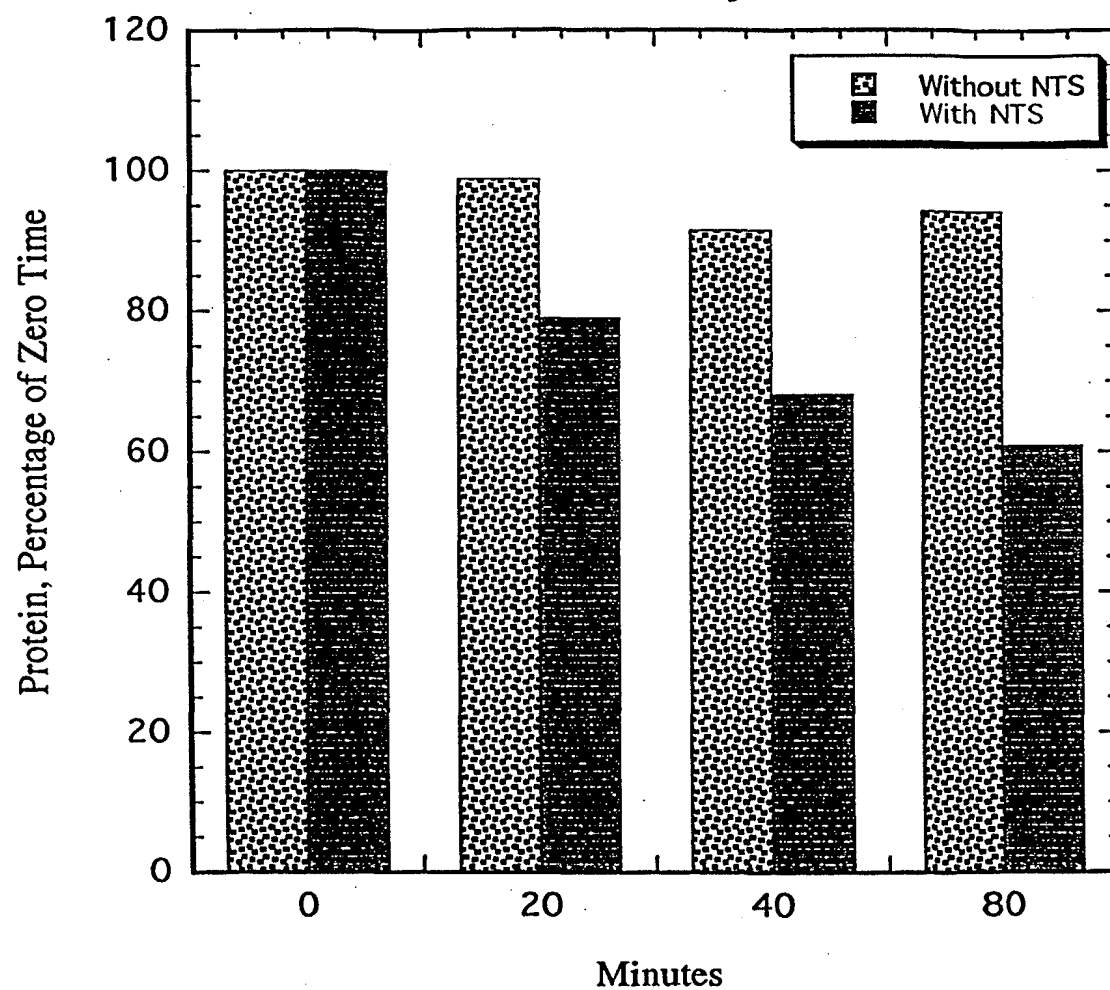
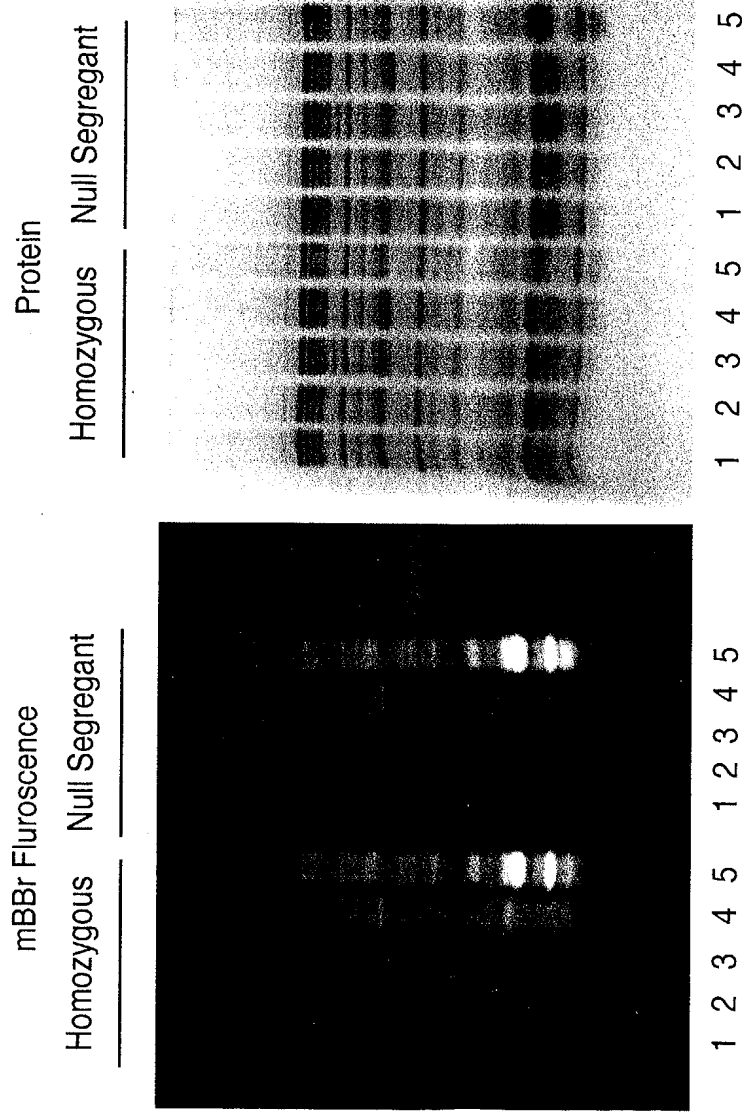


Figure 17

Effect of Thioredoxin Reduction on Digestion of
Wheat Glutenins by Pancreatin



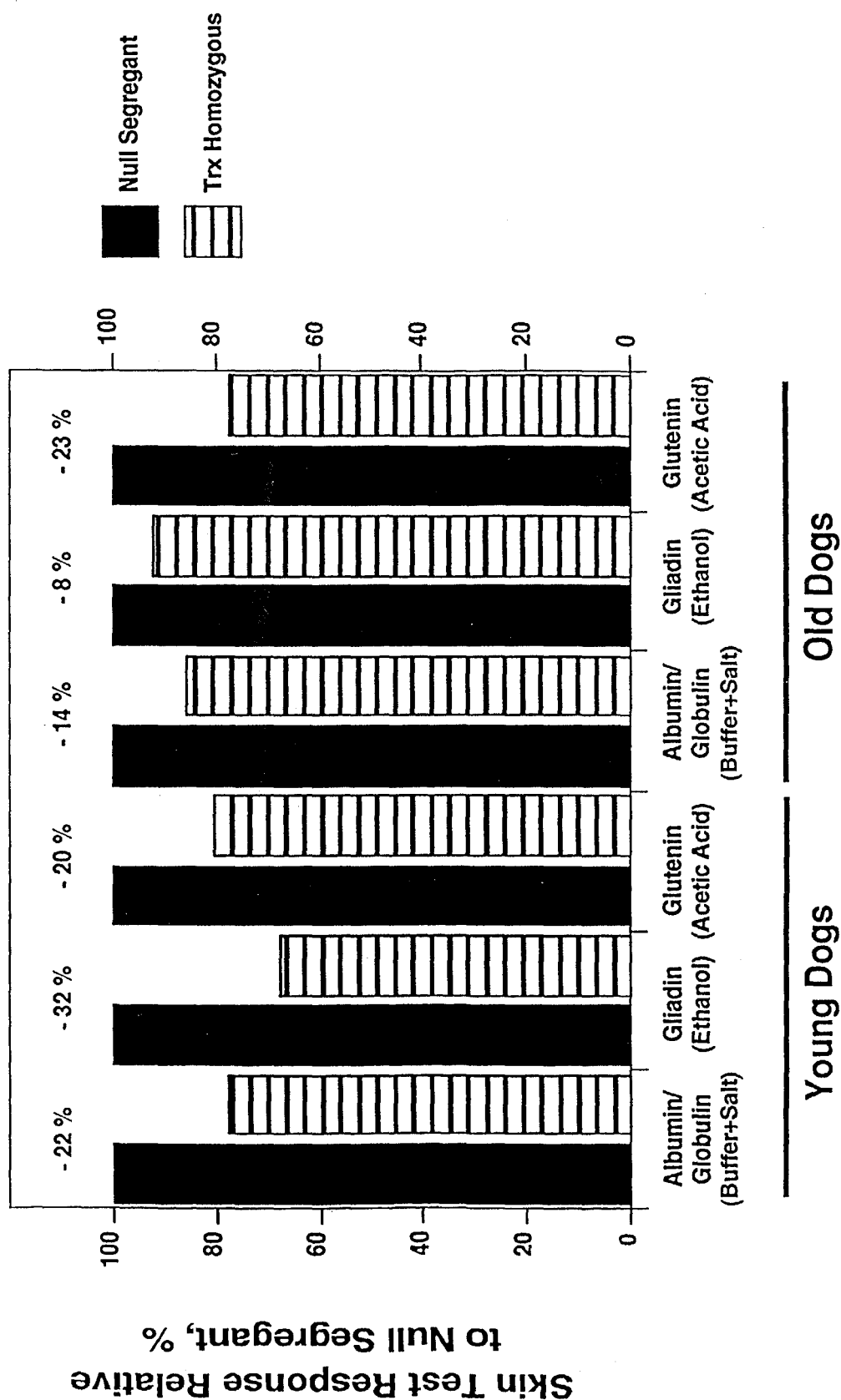
Effect of NTR on the Reduction of Proteins in Extracts of Wheat: Transgenic line Over-expressing Thioedoxin h vs. Null Segregant



1. Control
2. + NADPH
3. + NTR (*Arabidopsis*)
4. + NADPH & NTR
5. + DTT & boiled

Figure 18

Effect of Overexpressed Thioredoxin *h* on Allergenicity of Proteins from Wheat Grain



[illegible][illegible]

FIGURE 21

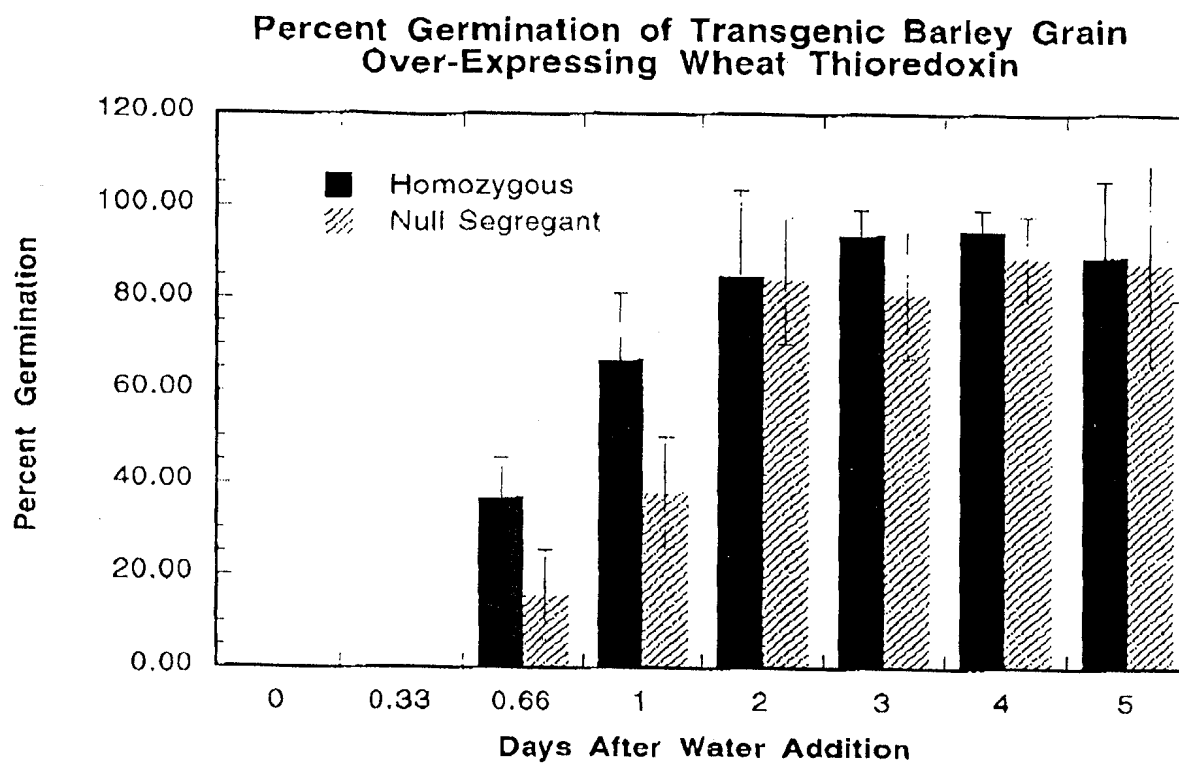
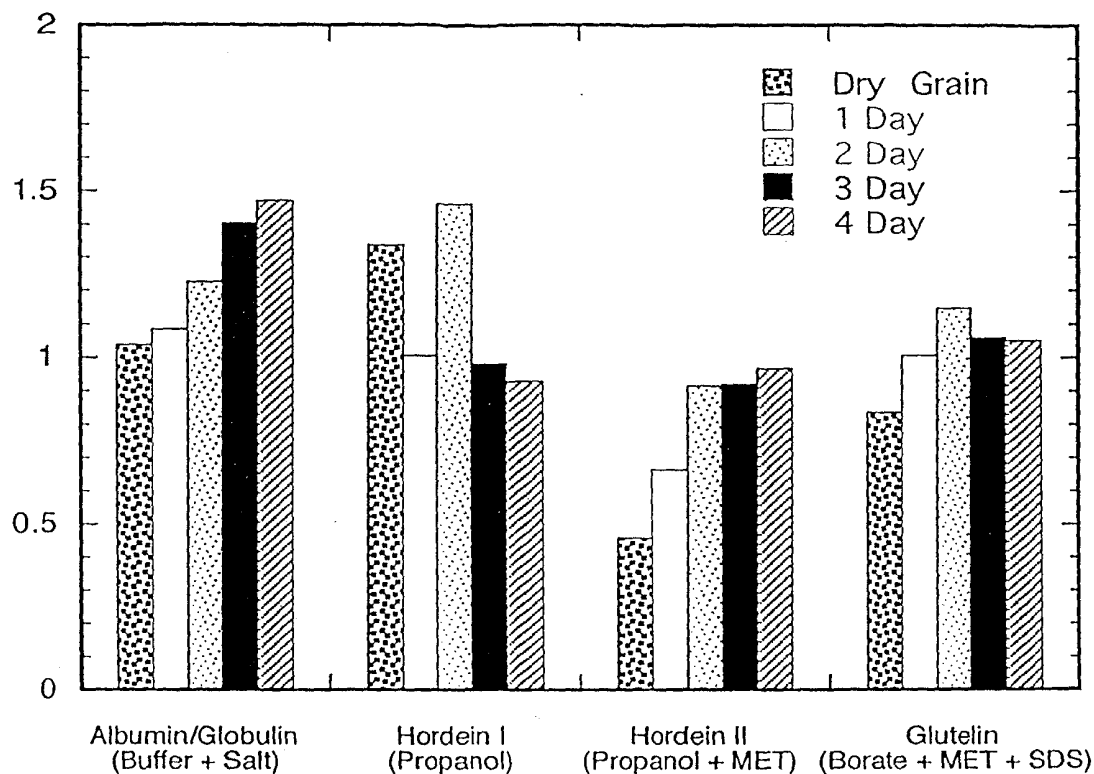


FIGURE 22

**Different Relative Redox Status of Protein Fractions
in Transgenic Barley Grain Over-Expressing
Wheat Thioredoxin h vs. the Null Segregant:
Dry and Germinating Grain**

006260 19960500
Ratio of Relative Reduction,
(Homozygous:Null Segregant)



Effect of Glucose-6-phosphate Dehydrogenase on the Reduction of Proteins in Extracts of Transgenic Wheat Grain Overexpressing Thioredoxin *h* in the Presence of

Glucose 6-phosphate and Arabidopsis NTR: +/- NTR

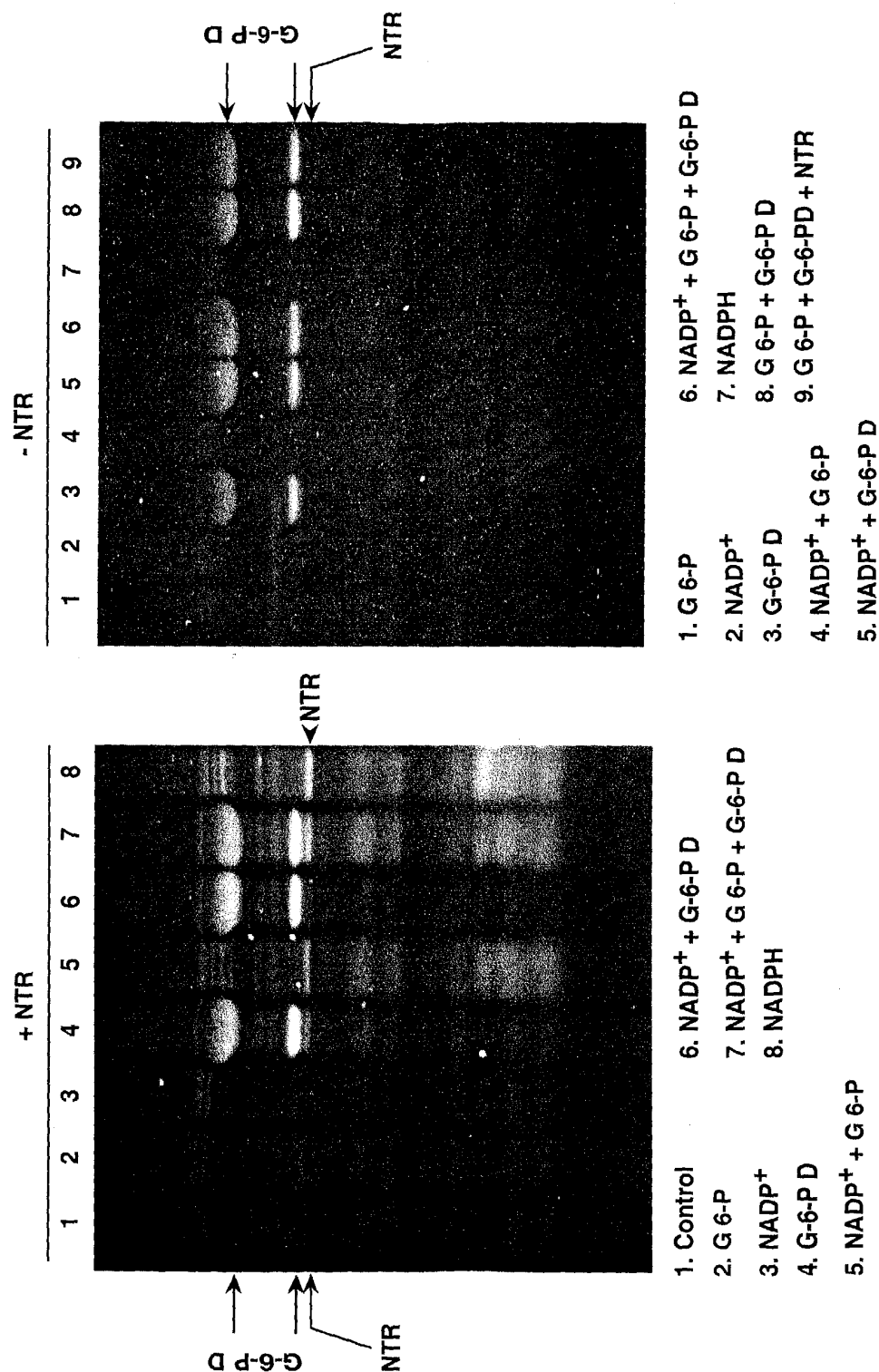


FIGURE 23

Effect of Glucose-6-phosphate Dehydrogenase on the Reduction of Proteins in Extracts of Null Segregant Derived from Wheat Grain Overexpressing Thioredoxin *h* in the Presence of Glucose 6- phosphate and Arabidopsis NTR: +/- NTR

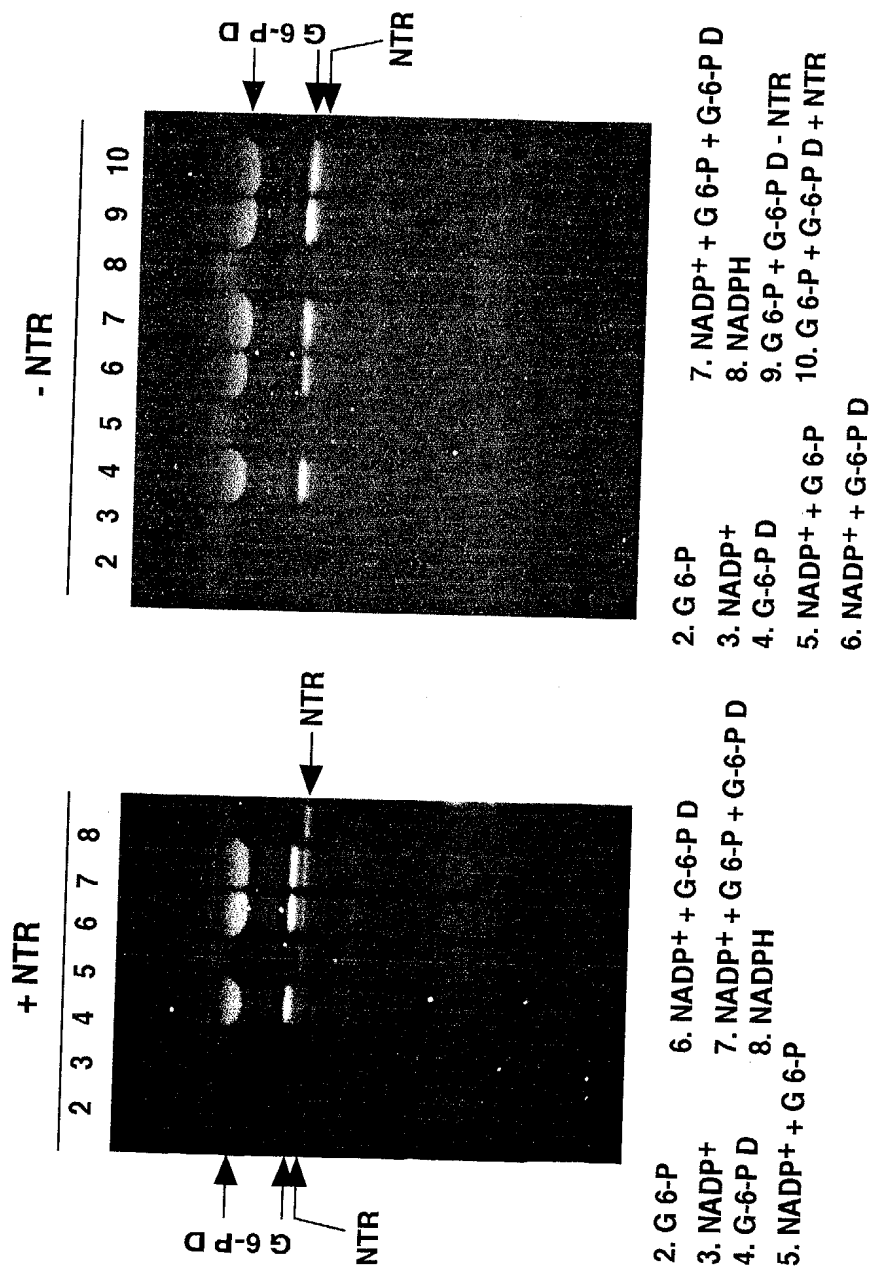


FIGURE 24